POPULATION GENETIC CONSEQUENCES OF FEEDING HABITS IN SOME FOREST LEPIDOPTERA

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ABSTRACT

By surveying variation at allozyme loci in several phytophagous lepidopteran species (Geometridae), we have tested two hypotheses about the relationship of genetic variation to environmental heterogeneity: (1) that allozyme polymorphisms may exist because of associations between genotypes and "niches" (different host plants, in this instance), and (2) that the overall genetic variation of a species is correlated with environmental heterogeneity (or breadth of the species' overall ecological niche).——Genetic differentiation among samples of oligophagous or polyphagous species taken from different host species was observed in one of three species, at only one of seven polymorphic loci. The data thus provide no evidence for pronounced genetic substructuring, or "host race" formation in these sexually reproducing species, although host plant-genotype associations in a parthenogenetic moth give evidence of the potential for diversifying selection.——In a comparison of allozyme variation in polyphagous ("generalized") and oligophagous ("specialized") species, heterozygosity appeared to be higher in specialized species, at all polymorphic loci but one. It is possible that this unexpected result arises from a functional relation between breadth of diet and genetic variation.

THIS paper is an attempt to test the applicability to allozyme loci of two hypotheses about the relationship of genetic variation to environmental heterogeneity.

The first hypothesis is that polymorphism may exist because different individuals in a population encounter or select different states of the environment. This idea, which we will call the hypothesis of multiple-niche polymorphism, was forcefully stated by DA CUNHA and DOBZHANSKY (1954), and first analyzed mathematically by LEVENE (1953). There have since been many theoretical treatments of this notion (review in FELSENSTEIN 1976), but few attempts to test it (review in HEDRICK, GINEVAN and EWING 1976).

This theory is important for several reasons. First, it is one of several competing hypotheses for the maintenance of polymorphism. Second, the widespread existence of multiple-niche polymorphism may lead to effects on breeding structure and linkage disequilibrium that could in turn affect a population's response to directional selection (see PROUT 1973; CHARLESWORTH and CHARLES-

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WORTH 1976; FELSENSTEIN 1976). Third, the existence of resource-specific genotypes could affect population distribution and abundance (*cf.*, ROUGHGARDEN 1972). Finally, multiple-niche polymorphism has been proposed as a possible intermediate stage in sympatric speciation.

Several laboratory and field studies suggest, to varying degrees, that particular "visible" polymorphisms could be maintained by diversifying selection (*e.g.*, DE SOUZA, DA CUNHA and DOS SANTOS 1970; GIESEL 1970; CLARKE, DICKSON and SHEPPARD 1963). However, to assess the importance of this mode of selection, we require surveys in which both "positive" and "negative" findings are reported. Other than allozyme studies, the only example is that of Fox (1975), who concluded that diversifying selection is rare and has only a transitory effect on meristic characters in a lizard, *Uta stansburiana*.

Our objective in this study is to determine if multiple-niche polymorphism is a common cause of allozyme variability. If so, one should be able to find differentiation in allozyme frequencies, among environmental patches within local populations. There have been several individual cases reported (*e.g.*, KOEHN, TURANO and MITTON 1973), but only two multiple-locus surveys for such differentiation. TAYLOR and POWELL (1977) found evidence for differences among microhabitat types at two of seven loci in a local population of *Drosophila persimilis*. Similarly, WILLIAMS, KOEHN and MITTON (1973) found such evidence at four of five loci in the unusual case of the American eel, the breeding population of which includes the entire coast of North America. Thus, the evidence so far suggests that multiple-niche polymorphism might be common at allozyme loci.

We report here on a survey for differentiation, with respect to host plant, within populations of three species of tree-feeding moths. The life histories of these insects follow closely several of the assumptions of Levene-type models. Each species is polyphagous, having more than one host plant, but individual larvae are relatively immobile and probably spend their entire feeding period on a single individual plant (see MATERIALS AND METHODS). Adults are vagile, short-lived and feed only on nectar.

Ecological studies of insect-plant relationships have shown that differences among hosts could potentially exert considerable disruptive selection on a population of phytophagous insects (*cf.*, DETHIER 1954). The possibility of polymorphism maintained by diet heterogeneity is given additional credibility by the observation that several mechanisms, including a tendency for adults to stay near the host, and possible "imprinting" of oviposition preference, may reduce inter-host gene flow (see DETHIER 1954).

Moreover, various authors since WALSH (1864) have proposed that the establishment of host-associated polymorphism could be an initial stage in speciation that might occur on a very local scale. If new insect species do commonly arise in this way, it should be possible to find evidence for the putative intermediate stages by a survey of populations that occupy two or more hosts. No such survey seems to have been carried out previously, although there is at least one probable case of such an intermediate stage (BUSH 1969).

The second hypothesis we have tested is that overall variability should be positively correlated with the degree of environmental heterogeneity that a population encounters. This theory would appear to be a logical extension of the hypothesis of multiple-niche polymorphism, as was first pointed out by DA CUNHA and DOBZHANSKY (1954). For two reasons, however, we would argue that this extension is not obvious. (1) As the number of niches (and of alleles corresponding to those niches) increases, the probability decreases that an allele will be distributed, before selection, into the niche to which it is adapted. Since nonrandom distribution of alleles into favorable niches greatly enhances the likelihood of stable polymorphism, it seems likely that the conditions for stable polymorphism must become more stringent as the number of niches and niche-specific alleles increases (*cf.*, LEWONTIN, GINZBURG and TULJAPURKAR's 1978 analysis of multiple alleles with heterozygous advantage). (2) If some large number of loci is potentially subject to disruptive selection, one might expect the evolution of some homeostatic mechanism that renders the whole organism less sensitive to environmental differences. An example of such an adaptation might be larger size, or increased developmental plasticity. Thus (although we are forced by the lack of appropriate theory to rely on heuristic arguments), we believe that, even if multiple-niche polymorphisms are common, one could reasonably predict any possible relationship or lack thereof between environmental and genetic variation.

Previous studies on the relationship of allozyme variability to environmental heterogeneity have yielded seemingly contradictory results. Two laboratory studies on Drosophila have shown a positive association (POWELL 1970; MCDONALD and AYALA 1974), but population sizes were not measured; moreover, this result might be an effect of the artificiality of the constant cage environment (SOULÉ 1973).

Studies on natural populations have yielded three reports of positive associations (BRYANT 1974a,b; LEVINTON 1973; STEINER 1977), one of no relationship (SOMERO and SOULÉ 1974), and one of an apparent negative relationship (summary by VALENTINE 1976 of work by several authors). In the studies that report a significant association, however, there is the possibility that the environmental gradient in question is confounded with other factors suspected to influence variability, such as population size or probability of recent directional selection (see SOULÉ 1975), while a real effect of environmental heterogeneity might be detectable only when the organisms compared are more closely related and ecologically similar than in the case reported by SOMERO and SOULÉ (1974).

We have tried to design a comparison that avoids these difficulties. We assayed the relationship between variability and breadth of diet in ten species of the geometrid subfamily Ennominae, which occur together in forests of Long Island, New York. These species have similar life histories, morphologies and geographic distributions. A readily apparent ecological difference among them, however, is their feeding habits: they range from strictly monophagous to broadly polyphagous. We shall later present evidence that population size and history are not confounded with breadth of diet.

It could be argued that a study such as ours would be pertinent only for loci functionally related to diet. We disagree with this view for two reasons. (1) For almost any enzyme, too little is known of *in vivo* functions (especially of interactions with other loci) and of linkage relationships to state with any assurance that its allele frequencies will be unaffected by host plant. An ecological variable such as food plant, which is known *a priori* to have potentially large and multifactorial effects on fitness, is a logical first place to assay the applicability of multiple-niche models to the persistent problem of variation revealed by electrophoresis. (2) If an important goal of population genetics is to explain evolutionary phenomena such as differences among species in rates of evolutionary change, it will be necessary to search for and explain differences among species in such overall genetic characteristics as the magnitude of genetic variation.

MATERIALS AND METHODS

The species we studied are listed in Table 1, with information on their feeding habits. All species were collected from their hosts as larvae. If not fully grown, they were raised in the laboratory on the host of origin and frozen at —70° upon reaching full size. Details of rearing procedures can be found in MITTER (1978).

TABLE 1

Feeding habits of lepidopteran species assayed

Species	Hosts*	Collections
1. *Itame pustularia*	*Acer rubrum*, red maple (*A. saccharum*, *A. saccharinum*)	Nissequogue 1974, 1975
2. *Patalene olyzonaria puber*	*Juniperus virginiana*, red cedar	Nissequogue 1975
3. *Heliomata cycladata*	*Robinia pseudoacacia*, black locust (*Gleditsia triacanthos*, Leguminosae)	Nissequogue 1975
4. *Lambdina pellucidaria*‡	*Pinus rigida*, pitch pine (other Pinus spp.)	Wading River 1974, 1975
5. *Lomographa vestaliata*†‡	*Prunus serotina*, black cherry (other rosaceous trees)	Nissequogue 1975
6. (*Probole nepiasaria*)‡	Vaccinium spp., highbush blueberry (*Rhododendron viscosum*, wild azalea; other Ericaceae)	Nissequogue 1975
7. *Epimecis hortaria*	*Liriodendron tulipifera*, tuliptree (Magnoliaceae); *Sassafras albidum*, sassafras (Lauraceae); *Lindera benzoin*, spicebush (Lauraceae)	Nissequogue 1974
8. *Tetracis cachexiata*	deciduous trees and shrubs of many families	Nissequogue 1975
9. *Melanolophia canadaria*	deciduous trees and shrubs of many families	Stony Brook 1974, 1975; Nissequogue 1975
10. *Melanolophia signataria*	deciduous trees and shrubs of many families	Stony Brook 1974, 1975

* Hosts in parentheses are rare on Long Island or are rarely used. Information derived from FORBES (1948) MCGUFFIN (1972), and our own observations (see MITTER 1978).

† See text.

‡ The genera *Lambdina*, *Lomographa*, and *Probole* have priority over the names *Therina*, *Bapta* and *Hyperetis*, respectively, which were used by FORBES (1948).

All but one species were collected during 1974 and 1975 in either or both of the woods on the campus of the State University of New York, Stony Brook, or the Nissequogue River State Park, Smithtown, New York, about 10 km west of Stony Brook. The Stony Brook site is a mixed hardwood stand, with a canopy primarily of oak, red maple and black birch, and an understory of dogwood and sassafras. The Nissequogue site contains a variety of habitats, including maple swamp with blueberry and spicebush understory, oak forest with dogwood and black cherry understory, and late successional fields dominated by red cedar and black locust. *Lambdina pellucidaria* was collected in pine-oak scrub near Wading River, New York, about 20 km east of Stony Brook. Detailed descriptions of these sites can be found in MITTER (1978).

The salient features of the life histories of the species studied are as follows. *Itame pustularia* is a bivoltine species whose larvae feed on maple from May to July; it overwinters as an egg. *Patalene olyzonaria puber* is a univoltine species whose larva feed exclusively on red cedar from early July to mid-August; some adults emerge in late August and September, and the rest overwinter as pupae and emerge the following June. *Heliomata cycladata* is univoltine; the larvae feed on locust in June and July, and the pupae overwinter. *Lambdina pellucidaria*, a univoltine species, feeds on pitch pine from June to early November and overwinters as a pupa; the adults fly in May. *Lomographa vestaliata*, also univoltine, feeds on rosaceous trees in July and August, overwinters as a pupa and emerges in late June. *Probole nepiasaria* is univoltine; it overwinters as a pupa, and the adults are active in May and June. The larvae feed on highbush blueberries and other ericaceous shrubs from June to September. It is closely related to *P. amicaria* and *P. alienaria*, which feed on dogwood (*Cornus florida*) and which occur in the locality where our specimens of *P. nepiasaria* were taken. Whether or not these species are distinct has been questioned by FORBES (1948), but other authors (*e.g.*, FERGUSON 1954) treat them as distinct species. Since our own experimentation has not resolved this issue (*e.g.*, we have obtained viable hybrids of *P. nepiasaria* and *P. alienaria* in the laboratory), we offer analyses of our data on overall genetic variation both with and without this species. *Epimecis hortaria* is a large species whose larvae feed on tulip tree, sassafras and spicebush from late May to early August. Most pupae overwinter, but about 10% of the adults emerge about two weeks after pupation; occasional small larvae are found in late August, indicating a small second brood. *Tetracis cachexiata* is univoltine and feeds on a wide variety of angiospermous trees and shrubs in late June and July. The pupae overwinter, and the adults fly in May and June. *Melanolophia canadaria* and *M. signataria* are both highly polyphagous, univoltine species whose larvae feed from late May until mid-July. About 10% of the adults emerge within two weeks of pupation and produce a very small second brood, but most of the pupae overwinter and emerge the following May. Except for *Lambdina pellucidaria*, which is found in pine barrens, all of these species are found in both late successional mesic habitats and more mature forests.

Electrophoresis was done in 11.5% horizontal starch gels. Larvae were homogenized in one volume of 0.1 M tris-HCl buffer containing 0.01 M β-mercaptoethanol, after the internal organs had been removed and discarded. The 15 loci initially surveyed (MITTER 1978) fell clearly into two groups, those that were never polymorphic except for one or two rare variants, and those that were quite variable in at least several species. Only the ten variable loci are included here, because the others do not contribute to the variation among species in overall variability. The variable loci, and the buffer systems in which they were assayed, are as follows. Phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), and malate dehydrogenase-1 (MDH-1) were run in the trismaleate buffer of SELANDER *et al.* (1971) for five hr at 100 v. Aminopeptidase-1 (AP-1; substrate *l*-leucyl-*l*-tyrosine) and aminopeptidase-2 (substrate *l*-leucyl-*l*-alanine) were run in the LiOH buffer of SELANDER *et al.* (1971) for five hr or longer, depending on the species (see MITTER 1978). Phosphohexose isomerase (PHI), mannose-6-phosphate isomerase (MI), esterase-2 (EST-2; substrate α-naphthyl acetate), and glutamate-oxaloacetate aminotransferase (GOT-1) were also run in the LiOH system, but with the gel buffer diluted by half. Adenosine deaminase (AD) was run in the phosphate buffer of SELANDER *et al.* (1971) for four hr at 120 v; for Melanolophia, the starch concentration was cut to 10%. Further details concerning these loci may be found in MITTER 1978.

The species assayed showed generally similar band patterns, with similar average mobilities, for all enzyme activities, and the same buffer generally gave the best resolution for all species for a given activity. These were the bases for the assumption of locus homologies in the analysis of overall variation. No data are available at some loci for some species, either because activity was too weak or because repeated attempts at resolution, using at least six of the buffer systems of SELANDER *et al.* (1971), were unsuccessful.

Breeding experiments with the gonochoristic/parthenogenetic geometrid *Alsophila pometaria* (MITTER and FUTUYMA 1977; unpublished data) have shown Mendelian transmission for *Phi*, *Ap-2*, and *Est-2*, and clonal inheritance for these loci plus *Pgm*, *Ap-1*, and *Ad* (for which no data on sexual broods were obtained). Our assumption that the variation reported in the present study is Mendelian is based on these results and on the general fit of observed genotypic proportions to those expected under Hardy-Weinberg equilibrium. For tests of goodness of fit, samples that did not differ in allele frequencies were combined and alleles were pooled until expected numbers were five or greater. Excluding *Est-2*, the 56 such tests performed showed only three cases of significant deviation from Hardy-Weinberg proportions, a result in accord with the hypothesis of Mendelian inheritance. At esterase-2, four of eight tests showed significant departures from Hardy-Weinberg, at least partly because of variation in enzyme activity (see LEIBENGUTH 1973a,b). For the sake of completeness and because there is some evidence that esterases may respond to selection by environmental toxins (ROCKWOOD-SLUSS, JOHNSTON and HEED 1973; TSAKAS and KRIMBAS 1970), we present our data on this locus, but do not include it in our analyses of genetic variation.

In order to determine whether larvae of *Epimecis hortaria* taken from different hosts differed in fitness on one of those hosts (see DISCUSSION), first-instar larvae were collected from tulip tree and sassafras at Nissequogue in 1973, and reared on sassafras. The cages were glass lamp chimneys as described by DROOZ (1970), and were kept at 21°, 70% RH, with foliage changed every other day. Individual pupae were sexed and weighed four days after pupation began.

We have used two measures of overall variation, corresponding to different mechanisms for the possible influence of diet heterogeneity on many loci simultaneously. A reasonable model, in light of the rarity of host-associated variation indicated by our data, is selection for high individual heterozygosity as proposed by SVED, REED and BODMER (1967), MILKMAN (1967) and KING (1967), whereby highly heterozygous genotypes are marginally overdominant across hosts. The appropriate measure under this hypothesis is the average heterozygosity ($\overline{H}$ of LEWONTIN and HUBBY 1966). On the other hand, if one views each locus as responding independently to diversifying selection and takes into account the fact that loci differ in average variability, it is appropriate to weight all loci equally in devising a summary statistic. Accordingly, we have also ranked the species for heterozygosity within each locus, and computed the average rank $\overline{R}$, over loci, for each species. Before the analyses were performed, missing values were replaced by the mean heterozygosity for that locus, across species.

Expected rather than observed heterozygosities were used so as to eliminate the effect of varying degrees of selection or of population mixing during the generations sampled. For *6-Pgd*, the expected heterozygosities have been halved, because this locus is sex-linked. When samples from different times, hosts, or localities did not differ in allele frequencies at a locus, they were combined for computation of $\overline{H}$. If samples from different times or localities differed, their heterozygosities were averaged. When samples from different hosts differed, heterozygosities were computed from unweighted averages of gene frequencies on different hosts. This was an attempt to simulate the effect of sampling at random from the entire population, with each host assumed to support an equal number of individuals. If strong host-specific differentiation were the rule, failure to sample all the hosts of nonmonophagous species would result in underestimation of the variation in these species. Since such differentiation was found to be rare (see RESULTS), this bias will be negligible.

The relation between genetic variation and breadth of diet was analyzed by Spearman's rank correlation, in which the species were assiged to three or four ranks, depending on whether or not *Probole nepiasaria* was included: (1) larval diet, one plant species (species 1 to 5, Table 1); (2) diet, several confamilial plant genera (*Probole nepiasaria*); (3) diet, two related plant

TABLE 2

Allele frequencies in larvae of Probole nepiasaria

Locus	Host	Alleles A	B	C	D	E	F	Others	*N*	χ^2(d.f.)†	*p*
Est	Vaccinium	0.13	0.02*	0.63	0.06*	0.06*	0.10	—	43	3.8(3)	>0.1
	Rhododendron	0.03	0.07*	0.64	0.05*	0.02*	0.17	—	29		
Mi	Vaccinium	0.14	0.35	0.45	0.03*	0.03*	0.01*	—	76	2.1(3)	>0.5
	Rhododendron	0.08	0.35	0.47	0.02*	0.03*	0.02*	—	30		
Ap-2	Vaccinium	0.03*	0.14	0.75	0.02*	0.06*	—	—	81	5.2(2)	>0.05
	Rhododendron	0.07*	0.07	0.73	0.07*	0.05*	—	—	30		
Pgm	Vaccinium	0.12*	0.37	0.47	0.04*	—	—	—	71	1.6(2)	>0.1
	Rhododendron	0.10*	0.45	0.45	0.00*	—	—	—	30		
Got	Vaccinium	0.12	0.11	0.76	—	—	—	—	74	2.0(2)	>0.1
	Rhododendron	0.13	0.05	0.82	—	—	—	—	30		
Mdh	Vaccinium	0.04*	0.91	0.05*	—	—	—	—	81	0.6(1)	>0.1
	Rhododendron	0.02*	0.95	0.03*	—	—	—	—	30		
Phi	Vaccinium	0.26	0.04*	0.37	0.04*	0.17	0.02*	0.09*	83	4.4(3)	>0.1
	Rhododendron	0.28	0.00*	0.48	0.02*	0.12	0.05*	0.05*	30		

* Alleles combined for χ^2 test.
† χ^2 test for differences in allele frequencies between hosts.

TABLE 3

Allele frequencies in larvae of Epimecis hortaria

Locus	Host	Alleles A	B	C	D	E	*N*	χ^2(d.f.)†	*p*
Pgm	Liriodendron	0.03*	0.76	0.19	0.02*	0.01*	58	0.53(2)	>0.5
	Lindera	0.01*	0.77	0.20	0.01*	0.00*	61		
Phi	Liriodendron	0.01*	0.03*	0.93	0.02*	0.00*	58	0.98(1)	>0.5
	Lindera	0.01*	0.05*	0.90	0.01*	0.03*	62		
Mi	Liriodendron	0.14*	0.00*	0.01*	0.84	0.02*	58	0.34(1)	>0.5
	Lindera	0.16*	0.01*	0.00*	0.82	0.02*	61		
Ap-1	Liriodendron	0.02*	0.64	0.26	0.08*	—	56	0.65(2)	>0.5
	Lindera	0.06*	0.59	0.30	0.07*	—	61		
Ap-2	Liriodendron	0.00*	0.94	0.05*	0.01*	—	58	0.76(1)	>0.1
	Lindera	0.01*	0.91	0.08*	0.00*	—	61		
Got-1	Liriodendron	0.99	0.01	—	—	—	58	2.7(1)‡	>0.05
	Lindera	0.96	0.04	—	—	—	58		
6-Pgd	Liriodendron	0.07	0.93	—	—	—	43	0.34(1)‡	>0.5
	Lindera	0.04	0.96	—	—	—	32		

* Alleles marked with asterisks were combined for χ^2 test.
† χ^2 test for differences in allele frequencies between hosts.
‡ χ^2 approximate because some expected values less than 5.

TABLE 4

Summary of results of allele frequency × host comparisons for six loci in Melanolophia canadaria*

Locus	Sample	Average N	χ^2(d.f.)	p
Mdh-1	†Nissequogue	49	0.00(2)	>0.90
	‡Stony Brook:			
	Cornus × Sassafras	36	0.79(1)	>0.10
Pgm	Nissequogue	47	0.81(2)	>0.50
	Stony Brook:			
	§ΣCornus × Quercus	106	2.29(1)	>0.10
	ΣCornus × Sassafras	103	0.34(1)	>0.50
Ap-2	Nissequogue	48	3.12(2)	>0.10
	Stony Brook:			
	ΣCornus × Sassafras	42	0.79(1)	>0.10
Got-1	Nissequogue	38	1.18(2)	>0.10
Ad	Nissequogue	42	2.64(2)	>0.10
	Stony Brook:			
	Cornus × Sassafras	38	6.60(3)	>0.05
Mi	Nissequogue	48	3.65(3)	>0.50
	Stony Brook:			
	ΣCornus × Sassafras	86	3.80(2)	>0.10
	ΣCornus × Quercus	86	2.30(2)	>0.10

* Complete data in MITTER (1978).

† Hosts sampled at Nissequogue were *Robinia pseudoacacia* (black locust), *Prunus serotina* (black cherry), and *Lindera benzoin* (spicebush).

‡ Hosts sampled at Stony Brook were Quercus = *Q.* cf. *rubra*, red oak; Sassafras = *S. albidum*, sassafras; Cornus = *Cornus florida*, flowering dogwood.

§ Sample from Quercus was taken in 1974; Sassafras sample is from 1975. Cornus was sampled in 1974 and 1975. For loci scored in both years, the Cornus samples were pooled because they did not differ in allele frequencies.

families (*Epimecis hortaria*); (4) diet, many plant families (species 8 to 10, Table 1). We also analyzed these data by a Mann-Whitney U test of differences between two groups of species, the "specialists" and "generalists," defined respectively as species that feed on a single plant family and species that feed on two or more plant families. This definition of specialist and generalist in Lepidoptera has proven interesting in other contexts (FUTUYMA 1976; WASSERMAN and MITTER 1978).

RESULTS

As can be seen from Tables 2 and 3, there is no evidence in *Probole nepiasaria* or *Epimecis hortaria* of differentiation related to the hosts sampled. The same is true of the Nissequogue population of *Melanolophia canadaria* (Table 4). Some evidence of differentiation does exist, however, for the Stony Brook collections of *M. canadaria*. Since the dogwood samples did not differ between years (Table 5), they were combined for separate comparisons to each of the other hosts sampled (to avoid confounding of years with hosts). Samples from dogwood differ from those taken from oak and sassafras at the *Phi* locus.

TABLE 5

Allele frequencies at the PHI locus in Melanolophia canadaria *larvae from different hosts*

Sample	Alleles A	B	C	D	E	Others	*N*	Comparison	χ^2(d.f.)	*p*
Niss 1975, Robinia	0.01*	0.08*	0.78	0.04*	0.01*	0.08*	38			
Niss 1975, Prunus	0.01*	0.03*	0.81	0.05*	0.01*	0.08*	69		0.79(2)	>0.5
Niss 1975, Lindera	0.00*	0.06*	0.76	0.09*	0.03*	0.03*	33			
SB 1974, Cornus	0.01	0.04	0.90	0.03	0.01	0.03	73			
								Cornus, 1974 × 1975†	4.4(2)	>0.1
SB 1974, Quercus	0.00*	0.08*	0.76	0.07	0.02*	0.04*	44			
								ΣCornus × Quercus	6.8(2)	>0.05
SB 1975, Cornus	0.01	0.02	0.84	0.09	0.01	0.03	46			
								ΣCornus × Sassafras	8.7(1)	>0.005
SB 1975, Sassafras	0.01*	0.04*	0.73	0.01*	0.03*	0.17*	37			

* Alleles combined for χ^2 test.
† Alleles A, B, E, Others combined for Cornus 1974 × 1975 comparison.

TABLE 6

Pupal weights of Epimecis hortaria *reared on sassafras**

Collected from	Males			Females			t_s	p
Tuliptree	0.39	(0.139)	18	0.46	(0.359)	13	3.2	>0.01
Sassafras	0.39	(0.094)	10	0.47	(0.188)	14	5.1	>0.001
t_s	0.0			0.14				
p	>0.9			>0.9				

* Values in each cell are mean (variance), sample size. Marginal values are t tests for differences between means.

Although there was a strong difference among the sexes, pupal weight in laboratory-reared *Epimecis hortaria* larvae showed no dependence on host of origin (Table 6).

Heterozygosity at each locus in each species is tabulated in Table 7. In Table 8, average heterozygosity ($\overline{H}$) and average rank in heterozygosity ($\overline{R}$) (see MATERIALS AND METHODS) are listed. We have analyzed these data both by performing a Spearman rank correlation of both $\overline{H}$ and $\overline{R}$ against ranked breadth of diet, and by a Mann-Whitney U test of average $\overline{H}$ and $\overline{R}$ of species assigned to two groups: "specialists" and "generalists." Both kinds of analyses have been performed both with and without *Probole nepiasaria*, for reasons noted in MATERIALS AND METHODS. The results of these analyses are presented in Table 9. The analysis of $\overline{R}$ consistently indicates that specialized species have greater levels of genetic variation than generalized species, a result we might not have anticipated from population genetic theory. However, average heterozygosity ($\overline{H}$) does not show a statistically significant relationship to breadth of diet, because this measure is dominated by highly heterozygous loci that do not vary very much among species. Table 10 demonstrates that all loci but one share in the overall trend of higher heterozygosity in specialized than in generalized species.

DISCUSSION

Since both the significant differences in allele frequencies among hosts involved a single population (*Melanolophia canadaria* on Cornus), it seems possible that this instance of genetic differentiation is a real effect, and not merely an artifact of the large total number of comparisons. If so, then one view of these results is that, since at least some evidence of within-population differentiation has been found in each of three attempts to survey for it (TAYLOR and POWELL 1977; WILLIAMS, KOEHN and MITTON 1973; present study), multiple niche polymorphism could contribute significantly to allozyme variation. On the other hand, our results indicate that a model of strongly host-dependent fitness or preference, at blocks of loci marked by allozymes, is unlikely to account for very much of the allozyme polymorphism in these insects. It appears that occupation of two or more hosts usually does not lead to extensive genetic substructuring.

TABLE 7

*Heterozygosity, by locus, in species of Ennominae**

	Probole nepiasaria	*Itame pustularia*	*Patalene puber*	*Heliomata cycladata*	*Lambdina pellucidaria*	*Lomographa vestaliata*	*Epimecis hortaria*	*Tetracis cachexiata*‡	*Melanolophia canadaria*	*Melanolophia signataria*‡
Got-1	0.37(104)	0.04(32)	0.00(39)	0.50(30)	0.11(26)	0.02(39)	0.04(110)	0.08(24)	0.34(104)	0.00(27)
Mdh-1	0.15(111)	0.17(32)	0.15(64)	0.15(32)	0.15(26)	0.02(39)	0.00(86)	0.13(22)	0.11(234)	0.00(27)
6-Pgd§	0.14(74)	0.17(28)	0.00(39)	—	0.14(62)	0.08(69)	0.04(74)	0.00(23)	0.00(75)	0.00(27)
Ap-1	—†	0.24(32)	0.35(63)	0.37(29)	0.41(28)	—	0.54(119)	0.00(22)	0.00(32)	—
Ap-2	0.43(111)	0.57(147)	0.47(65)	0.03(30)	0.53(97)	0.43(52)	0.15(119)	0.47(24)	0.09(228)	0.04(27)
Est-2	0.51(72)	0.46(129)	0.43(24)	0.53(23)	0.31(86)	0.71(68)	—	0.36(24)	0.59(57)	—
Pgm	0.62(101)	0.33(147)	0.25(65)	0.44(53)	0.32(122)	0.63(78)	0.37(119)	0.32(24)	0.15(266)	0.52(27)
Phi	0.74(113)	0.40(278)	0.17(65)	0.35(55)	0.52(125)	0.67(104)	0.15(120)	0.18(24)	0.39(340)	0.52(23)
Mi	0.64(106)	0.09(32)	0.38(64)	0.10(54)	0.35(79)	0.35(13)	0.29(119)	0.48(23)	0.42(352)	0.67(27)
Ad	—	—	0.48(52)	—	—	—	0.10(30)	—	0.50(201)	—

* Values are expected heterozygosity, calculated as in text; numbers in parentheses are numbers of individuals examined. Raw data in MITTER (1978).

† Blank cells indicate no data available.

‡ *Tetracis cachexiata* was collected from *Acer rubrum, Robinia pseudo-acacia, Prunus serotina,* and *Lindera benzoin*; *Melanolophia signataria* was taken from *A. rubrum, R. pseudo-acacia, P. serotina, Cornus florida,* and *Quercus alba.*

§ Expected heterozygosities have been halved because locus is sex-linked.

TABLE 8

Overall variation in relation to breadth of diet

	$\bar{H}$	$\bar{R}$, With Probole	$\bar{R}$, Without Probole	Mean N/Locus
Species feeding on a single plant family				
Itame pustularia	0.26	4.94	4.33	95
Patalene polyzonaria	0.25	6.00	5.39	54
Heliomata cycladata	0.26	5.39	4.06	38
Lambdina pellucidaria	0.32	4.17	3.56	72
Lomographa vestaliata	0.31	5.22	4.50	58
(*Probole nepiasaria*)	0.41	3.39	—	99
Mean	0.30	4.85	4.37	
Species feeding on two or more plant families				
Epimecis hortaria	0.19	7.00	6.11	55
Tetracis cachexiata	0.22	6.33	5.50	24
Melanolophia signataria	0.26	6.33	5.44	27
Melanolophia canadaria	0.22	6.22	5.44	191
Mean	0.22	6.47	5.62	

TABLE 9

Analyses of relation between genetic variation and breadth of diet

		Mann-Whitney U test U	Mann-Whitney U test $p\leq$	Rank correlation r_s	Rank correlation $p\leq$
With Probole	$\bar{H}$	21	0.10	0.498	0.20
With Probole	$\bar{R}$	24	0.01	0.648	0.05
Without Probole	$\bar{H}$	17	0.20	0.606	0.10
Without Probole	$\bar{R}$	20	0.02	0.783	0.01

TABLE 10

Mean heterozygosity $\bar{H}$ *of species by diet group, at each locus*

Locus	Species feeding on one plant family With *P. nepiasaria*	Species feeding on one plant family Without *P. nepiasaria*	Species feeding on two or more plant families
Got-1	0.17	0.13	0.12
Mdh-1	0.13	0.13	0.06
6-Pgd	0.11	0.10	0.01
Ap-1	0.33	0.35	0.21
Ap-2	0.41	0.41	0.25
Est-2	0.49	0.49	0.42
Pgm	0.43	0.39	0.34
Phi	0.48	0.42	0.31
Ad	0.32	0.37	0.30
Mi	0.32	0.27	0.47

One might argue, of course, that the changes involved in the origin of so-called "host races" are limited to the characters directly involved in adaptation to the host, and thus might not be reflected by allozyme markers. We have one piece of evidence on this point. In the oligophagous *Epimecis hortaria*, for which we have no evidence of host-related allozyme differences, it appears that larvae from tulip tree are just as well adapted to sassafras (Table 6), as measured by pupal weight (strongly correlated with female fecundity in Lepidoptera [*e.g.*, BECKWITH 1976; DROOZ 1965]). From these data, and from our more general but unsystematic observation that larvae of all geometrids studied can easily be transferred between hosts characteristic of the species as a whole, we suggest that host-associated differentiation is not the rule in these insects.

Our results raise the following question: Is the apparently low incidence of host-related differentiation a result of high migration between hosts and recombination preventing the accumulation of host-adapted blocks of loci, or is the disruptive selection imposed by diet heterogeneity less important than we originally supposed? We have been able to answer this question partially by studying the parthenogenetic race of the geometrid *Alsophila pometaria* (MITTER and FUTUYMA 1977), in which the larvae are dispersed by wind and the females are wingless. At our Nissequogue site and elsewhere, the frequencies of certain parthenogenetic genotypes differ consistently between adjacent stands of oak and maple (MITTER *et al.*, 1979). Thus, strong host-specific selection can be detected in an extreme case of reduced recombination. Since most genotypes are virtually absent from extensive stands of the "wrong" host and since the diversity of clones cannot be attributed to heterosis, host differences must in this case be sufficient to maintain genetic (inter-clonal) variation in the population as a whole. These data therefore suggest that diet heterogeneity can impose strong diversifying selection on a population, and that the infrequency of host-associated polymorphism observed in our sexual species could be due to factors, such as recombination, that militate against the formation of associations between host plant and allozyme-marked blocks of genes.

Our analysis of overall variability suggests that, in these insects, the relationship between genetic variation and environmental variability (as measured by diet breadth) may be negative. It is clearly desirable to study more species, since the statistical significance (although not the qualitative direction) of our result depends on how genetic variation is measured and compared. Nevertheless, we think it important to attempt to explain this observation.

This trend could arise if "specialists" have larger population sizes than "generalists." We have no data on population sizes for many of these species. However, FUTUYMA and GOULD (1979) have measured the density of larvae of each of many lepidopteran species on each species of woody host plant in a forest north of New York City. In these collections, which include some of the same species of geometrids reported in the present study, there was no evidence of any relationship between abundance and breadth of diet.

It has been argued that colonization and speciation events reduce variability through sampling error and directional selection (SOULÉ 1975). However, there

is no reason to suppose that generalized feeders have experienced more such events than specialists. Large groups of very similar species (the only evidence available, in the absence of cladistic studies, of relatively frequent speciation) are not more common among generalized feeders in the Lepidoptera; if anything, the opposite is true (*cf.*, FORBES 1948; DETHIER 1954).

Thus, we think it unlikely that our results can be explained on the basis of population size or history. They are probably due to a difference in the regimes of selection and/or migration experienced by species that differ in breath of diet.

WASSERMAN and MITTER (1978) have found that generalists (as defined above) are significantly larger than specialists in the Ennominae and in forest moths generally. This might cause specialists to have lower vagility (although we are aware of no data on this question). The spatial distributions of specialists may also be less continuous, reflecting the distribution of suitable hosts. One plausible explanation for our observation, therefore, is that specialists are more likely to develop polymorphism in response to local variation in selection coefficients, due to their lower rates of migration between environmental "patches."

A second possibility is that, in accord with arguments presented above, the potentially great environmental uncertainty faced by generalists may have led to the evolution of some "homeostatic" mechanism that reduces the environmental variance "perceived" by most loci. If, conversely, specialized species lacked such homeostatic mechanisms, variation in chemical or other properties within a single host species could maintain genetic variation (*e.g.*, by marginal overdominance) that would not be maintained in more generalized species. This hypothesis is consistent with the observation that generalists are larger than specialists, since large size is known to buffer organisms against environmental variation (*cf.*, BONNER 1965). Moreover, KRIEGER, FEENY and WILKINSON (1971) report that mixed function oxidase activity is higher in generalized than in specialized species of Lepidoptera; this "general-purpose" detoxifying activity may be a mechanism by which generalists avoid the need for special adaptations to each of the many plant defense compounds they may encounter.

We are not the first to suggest that adaptation to environmental heterogeneity may entail changes in physiological buffering systems that reduce levels of genetic polymorphism (*cf.*, THODAY 1953; JAIN and MARSHALL 1967; SELANDER and KAUFMAN 1973). We emphasize, however, that although the apparent differences that we have found between levels of heterozygosity in generalized and specialized species are consistent with this hypothesis, they are consistent with other hypotheses as well. Nevertheless, if a consistent relationship between environmental variation and genetic variation emerges from this and future studies, it may well be of a different form than has generally been supposed.

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